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Characterization of inhibitory effects of the potential therapeutic inhibitors, benzoic acid and pyridine derivatives, on the monophenolase and diphenolase activities of tyrosinase

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ABSTRACT

Objective(s): Involvement of tyrosinase in the synthesis of melanin and cell signaling pathway has made it an attractive target in the search for therapeutic inhibitors for treatment of different skin hyperpigmentation disorders and melanoma cancers.

Materials and Methods: In the present study, we conducted a comprehensive kinetic analysis to understand the mechanisms of inhibition imposed by 2-amino benzoic acid, 4-amino benzoic acid, nicotinic acid, and picolinic acid on the monophenolase and diphenolase activities of the mushroom tyrosinase, and then MTT assay was exploited to evaluate their toxicity on the melanoma cells.

Results: Kinetic analysis revealed that nicotinic acid and picolinic acid competitively restricted the monophenolase activity with inhibition constants (K_i) of 1.21 mM and 1.97 mM and the diphenolase activity with K_is of 2.4 mM and 2.93 mM, respectively. 2-aminobenzoic acid and 4-aminobenzoic acid inhibited the monophenolase activity in a non-competitive fashion with K_is of 5.15 µM and 3.8 µM and the diphenolase activity with K_is of 4.72 µM and 20 µM, respectively.

Conclusion: Our cell-based data revealed that only the pyridine derivatives imposed cytotoxicity in melanoma cells. Importantly, the concentrations of the inhibitors leading to 50% decrease in the cell density (IC_{50}) were comparable to those causing 50% drop in the enzyme activity, implying that the observed cytotoxicity is highly likely due to the tyrosinase inhibition. Moreover, our cell-based data exhibited that the pyridine derivatives acted as anti-proliferative agents, perhaps inducing cytotoxicity in the melanoma cells through inhibition of the tyrosinase activities.

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Introduction

Tyrosinase (EC 1.14.18.1) is a copper-containing bifunctional enzyme, widely distributed in mammals, plants and micro-organisms (1). This enzyme catalyzes hydroxylation and oxidation of mono-phenols and diphenols, respectively (2). In fact, it catalyzes the hydroxylation of tyrosine to form 3,4-dihydroxyphenylalanine (L-DOPA), and L-DOPA to DOPA quinine (3). Quinones, in turn, develop chemically to melanins and other polyphenolic compounds (4).

Engagement of tyrosinase in important cell signaling pathways has made the enzyme an attractive target in the search for therapeutic inhibitors for prevention and treatment of different disorders including skin hyperpigmentation and cancers (5, 6). In addition, inhibition of tyrosinase with various kinds of inhibitors has been a useful tool for gaining better understanding of the mechanism of action of the enzyme (7, 8). In the past decade, a large number of compounds such as benzaldehyde and benzoate derivatives have been identified that inhibit the enzyme activity (9–16). It is known that the aldehyde group in the benzaldehyde derivatives react with functional groups including hydroxyl, amino and sulfhydryl group (9, 10). Previous studies have indicated that benzaldehyde inhibitors restrict the enzyme activity by forming a Schiff base with a primary amino group in the enzyme structure (14, 15). It has also been suggested that benzoate inhibits tyrosinase by a copper chelating mechanism (16). It is thought that

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